

Research Note

Effects of Supplemental Dietary Phytase and 25-Hydroxycholecalciferol on the Performance Characteristics of Commercial Layers Inoculated Before or at the Onset of Lay with the F-Strain of *Mycoplasma gallisepticum*^{1,2}

E. D. Peebles,^{*3} S. L. Branton,[†] M. R. Burnham,^{*} S. K. Whitmarsh,^{*} and P. D. Gerard[‡]

^{*}Department of Poultry Science, Mississippi State University, Mississippi State, MS 39762; [†]Poultry Research Unit, Agricultural Research Service, USDA, Mississippi State, MS 39762; and [‡]Department of Applied Economics and Statistics, Clemson University, Clemson, SC 29634

ABSTRACT The effects of dietary supplementation with phytase and 25-hydroxycholecalciferol on the performance characteristics of commercial layers that were inoculated prelay (12 wk of age) or at the onset of lay (22 wk of age) with F-strain *Mycoplasma gallisepticum* were assessed. Experimental layer diets, which included a basal control diet or the same diet supplemented with 0.025% phytase and 25-hydroxycholecalciferol, were fed from 20 through 58 wk of age. Weekly and total egg production were determined from 22 through 58 wk, and egg weight and various internal egg and eggshell quality characteristics were examined at 34, 50, and 58 wk of age. F-strain

M. gallisepticum inoculation decreased egg production at the beginning of lay (wk 22 and 23) but increased post-peak lay at wk 45. However, there were no treatment effects of any kind on total egg production, egg weight, or any of the internal egg and eggshell characteristics examined during lay. In conclusion, dietary supplementation with phytase and 25-hydroxycholecalciferol did not affect layer performance or interact with the effects of F-strain *M. gallisepticum* inoculation; however, F-strain *M. gallisepticum* inoculation resulted in a shift in egg production from wk 22 to 45 without having an overall effect on total egg production.

Key words: F-strain *Mycoplasma gallisepticum*, inoculation, *Mycoplasma gallisepticum*, phytase, 25-hydroxycholecalciferol

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INTRODUCTION

The F-strain of *Mycoplasma gallisepticum* has been shown to be effective in minimizing egg production (EP) losses in commercial layers if given in an inoculation before they are exposed to more virulent field strains of *M. gallisepticum* (Luginbuhl et al., 1976). Nevertheless, Burnham et al. (2002) reported that inoculation of commercial layers with F-strain *M. gallisepticum* at 12 wk of age delayed onset of lay and decreased total EP. Because F-strain *M. gallisepticum* is known to be able to colonize the liver (Sahu and Olson, 1976), losses in EP after an F-strain *M. gallisepticum* inoculation may be due to its disruption of yolk lipid synthesis in the liver.

It is of current interest to determine whether a combination of supplemental dietary phytase (PHY) and 25-hydroxycholecalciferol (25-D₃) might help to alleviate the negative impact of F-strain *M. gallisepticum* on EP. In support of this supposition, Carlos and Edwards (1998) observed that the supplementation of basal layer diets with PHY or 1,25-dihydroxycholecalciferol, or their combination prevented a rapid decrease in EP because of an *M. gallisepticum* infection. They did not identify the strain of *M. gallisepticum* involved, but it is presumed to have been a field strain. The addition of PHY was also shown by Carlos and Edwards (1998) to have a positive effect on BW, tibia bone ash, and plasma Ca. Therefore, the current objective was to determine the effects of F-strain *M. gallisepticum* inoculations given prelay (12 wk of age) and at onset of lay (22 wk of age) in commercial layers fed either a basal control diet or the same diet supplemented with PHY and 25-D₃ on EP, egg weight (EW), eggshell quality, and internal egg characteristics.

MATERIALS AND METHODS

Bird Management

All 3 trials were conducted under an approved USDA Animal Care and Use protocol. In the pretreatment pullet

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³Corresponding author: dpeebles@poultry.msstate.edu

Table 1. Egg production (% of hen-day egg production) at 22, 23, and 45 wk of age in commercial layers that were sham-inoculated or F-strain *Mycoplasma gallisepticum*-inoculated^{1,2}

Inoculation treatment	Week of age		
	22	23	45
	(%)		
Sham	83.8 ^a	94.0 ^a	75.8 ^b
F-strain <i>M. gallisepticum</i>	76.8 ^b	90.1 ^b	79.2 ^a

^{a,b}Means within a column with no common lower case superscript differ ($P \leq 0.05$).

¹n = 36 replicate units for calculation of mean within each hen age and diet treatment group.

²SEM based on pooled estimate of variance = 1.09.

period, chickens (Hy-Line W-36) were vaccinated, basal starter and grower diets were formulated, and procedures and materials used to test for the presence of *M. gallisepticum* and *Mycoplasma synoviae* were performed as specified by Peebles et al. (2003). Beginning at 12 wk of age in each of 3 trials, 240 birds were randomly placed in individual cages in a commercial caged layer facility. One end of the facility housed sham-inoculated birds (120), and the other end housed F-strain *M. gallisepticum*-inoculated birds (120). In each end, birds were inoculated at 12 or 22 wk of age, and beginning at 20 wk and continuing through 58 wk of age, birds were fed experimental diets that included either a basal control diet or the same diet supplemented with PHY and 25-D₃. Further details of pullet housing, inoculation materials and procedures, and descriptions of the experimental layer diets and their allotment are given by Peebles et al. (2007a).

Data Collection

Egg production was recorded daily and analyzed weekly from 22 through 58 wk and was expressed as percentage of hen-day EP. Data from all 3 trials were used to determine EP from 22 through 53 wk, whereas data from 2 of the 3 trials were used to determine EP from 54 through 58 wk. Therefore, EP data for wk 22 to 53 and for wk 54 to 58 were analyzed separately. Furthermore, total or cumulative EP from 22 through 58 wk was determined for the same 2 trials as were used for determination of weekly percentage hen-day EP from 54 through 58 wk. Total EP was expressed as the percentage

of total hen EP and was calculated as total daily numbers of eggs produced as a percentage of the total daily numbers of hens for each replicate group over the entire 22- to 58-wk production period.

At wk 34, 50, and 58, EW (g); percentages of eggshell, albumen, and yolk weights; eggshell weight per unit of surface area (SWUSA; mg/cm²); and percentage of yolk lipid and moisture contents (% of fresh yolk sample weight) were determined. At least 10 eggs were collected from each replicate unit (Buss, 1984) on a given day. If necessary, more were collected the following day of the same week. Determinations were made on the same day that eggs were collected. Eggshell weight was determined according to the procedure described by Brake et al. (1984). Relative eggshell (dried shell plus membranes), albumen (fresh), and yolk (fresh) weights were expressed as percentages of total EW. The SWUSA of eggs was determined by using the following formula: SWUSA = [eggshell weight (mg)/eggshell surface area (cm²)], where eggshell surface area = $3.9782 \times EW^{0.7056}$ (Carter, 1975). For analysis of yolk moisture content, fresh yolk samples were dried according to the procedure of Peebles et al. (1999), and for analysis of yolk lipid concentration, lipid was extracted according to the procedure previously described by Bligh and Dryer (1959) and as modified by Latour et al. (1998).

Statistical Analysis

A randomized complete block experimental design, with trial as a block, was used. The data from all 3 trials were pooled and then analyzed together. Weekly EP from 54 through 58 wk of age and total EP data were obtained from only 2 of the 3 trials. Therefore, weekly EP data from 54 through 58 wk were analyzed separately from weekly EP data from 22 through 53 wk of age. A split-plot treatment structure was used, with inoculation type as the whole-plot factor and age of inoculation and diet as subplot factors. Weekly EP; EW; percentages of eggshell, albumen, and yolk weights; SWUSA; and percentages of yolk lipid and moisture concentration data were subjected to a repeated measures analysis to account for the fact that the same experimental units were observed over multiple age periods. The fixed effects of dietary treatment (control vs. PHY and 25-D₃), age of inoculation (12 vs. 22 wk), type of inoculation (sham vs. F-strain *M. gallisepticum*),

Table 2. Mean total egg production (% of hen egg production) in commercial layers that were sham-inoculated (sham) or F-strain *Mycoplasma gallisepticum*-inoculated at 12 or 22 wk of age and that were fed unsupplemented basal diets (basal) or diets supplemented with phytase and 25-hydroxycholecalciferol (PHY/25-D₃)^{1,2,3}

Diet	Sham				F-strain <i>M. gallisepticum</i>			
	12 wk		22 wk		12 wk		22 wk	
	Basal	PHY/25-D ₃	Basal	PHY/25-D ₃	Basal	PHY/25-D ₃	Basal	PHY/25-D ₃
Egg production	71.3	76.5	72.0	76.3	72.5	75.2	73.0	73.7

¹n = 24 replicate units for calculation of mean within each treatment group.

²SEM based on pooled estimate of variance = 3.31.

³No significant differences among means were noted.

hen age, and their interactions were tested. Total EP data were analyzed by 1-way analysis. Trial and interactions between fixed effects and trial were considered random effects. Replicate means of those parameters for which subsamples were taken were used in all data analyses. Least-squares means were compared in the event of significant global effects (Steel and Torrie, 1980). All data were analyzed by using the MIXED procedure of SAS software (SAS Institute, 2000). Statements of significance were based on $P \leq 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

As indicated in the companion article by Peebles et al. (2007a), using these same birds and experimental protocol, no significant differences were demonstrated between the treatments (diet, inoculation type, or inoculation age) for cumulative bird mortality or reproductive organ histopathological lesion scores. Likewise, Burnham et al. (2002) reported that the inoculation of layers with F-strain *M. gallisepticum* at 12 wk in each of 2 trials did not significantly affect mortality.

In the current study, there was a significant hen age by inoculation type interaction for weekly EP from 22 through 53 wk ($P \leq 0.0001$). Significant inoculation type treatment effects on EP were observed only at wk 22, 23, and 45. Inoculation type treatment means for only those 3 time periods are specifically shown in Table 1. Inoculation with F-strain *M. gallisepticum* (across the 12- and 22-wk inoculation ages) decreased EP at wk 22 and 23, whereas at wk 45, EP was increased by F-strain *M. gallisepticum* inoculation. However, no noted effects on weekly EP were attributable to time of inoculation or diet.

Similar to the weekly EP results in this study, Burnham et al. (2002) reported that onset of lay was delayed by approximately 1 wk in layers inoculated with F-strain *M. gallisepticum* at 12 wk of age in 2 trials. Nevertheless, total EP was significantly reduced by the 12-wk F-strain *M. gallisepticum* inoculation in only 1 of the 2 trials. In addition, across 2 of the trials in the current study, F-strain *M. gallisepticum* inoculation had no effect on total EP from 22 through 58 wk. This would indicate that the increase in weekly EP at wk 45 compensated for the decrease in EP at 22 and 23 wk because of F-strain *M. gallisepticum* inoculation. Although there were no treatment effects on total EP, mean total EP data for each of the treatment groups are provided in Table 2 for reference. Differences in the results of this study and those of Burnham et al. (2002) may have been partly due to the inclusion of both 12- and 22-wk F-strain *M. gallisepticum* inoculations in the present study. In addition, birds in the Burnham et al. (2002) report were housed in negative pressure biological isolation units, and those in this study were housed in a caged layer facility.

As described in the companion article by Peebles et al. (2007a), the BW of sham-inoculated control birds were significantly reduced by dietary PHY and 25-D₃ supplementation, whereas dietary treatment had no effect in F-strain *M. gallisepticum*-inoculated hens (diet by inocula-

tion type interaction). The bases for the effect of the supplemented diet in control birds and the loss of the effect in F-strain *M. gallisepticum*-inoculated birds was not clear to Peebles et al. (2007a), but did indicate that F-strain *M. gallisepticum* negated the depressing effects of the supplemented diet on BW. Nevertheless, neither weekly nor total EP in the present study was affected by diet. Furthermore, across diet and age of inoculation, Peebles et al. (2007a) reported that F-strain *M. gallisepticum* did not affect BW, whereas F-strain *M. gallisepticum* was found to affect EP at wk 22, 23, and 45 in the current study. This would suggest that the changes in EP in response to treatment were not associated with the changes in BW.

There were no treatment effects of any kind on EW or the internal egg and eggshell characteristics examined. Using the same birds as in this study, Peebles et al. (2007b) reported that diet and time of inoculation affected the reproductive organ characteristics. Based on these earlier results, inoculation and dietary treatment main and interactive effects on EW and internal egg and eggshell quality would be expected. In addition, dietary effects on EP would also have been anticipated. Conversely, only the inoculation of F-strain *M. gallisepticum* (before or at the onset of lay) caused a decrease in EP at wk 22 and 23, and caused an increase in EP at wk 45.

In conclusion, in terms of EP and the eggshell and internal quality of the eggs laid, dietary supplementation with PHY and 25-D₃ did not affect layer performance or interact with the effects of F-strain *M. gallisepticum* inoculation; however, F-strain *M. gallisepticum* inoculation resulted in a shift in EP from wk 22 to 45 without having an overall effect on total EP.

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